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(54) Title: ACETYLCHOLINESTERASE INHIBITORS IN COMBINATION WITH MUSCARINIC AGONISTS FOR THE TREAT- MENT OF ALZHEIMER'S DISEASE (57) Abstract New compositions of matter and a method for treating bodily disorders involving cholinergic hypofunction such as Alzheimer's disease in a mammal are disclosed. The compositions comprise a combination of an acetylcholinesterase inhibitor and a muscarinic agonist. The method comprises administration of the combination to a mammal. The instant invention demonstrates that the combination of an acetylcholinesterase inhibitor and a muscarinic agonist can be safely administered, that doses of each agent which by themselves showed no activity yielded positive responses and minimal side effects in combination, and that the active dose range for both agents could be widened when used in combination. These results imply that the combined treatment may eliminate the need to individually titrate doses and also increase the separation between efficacy and adverse events.		

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ACETYLCHOLINESTERASE INHIBITORS IN COMBINATION WITH
MUSCARINIC AGONISTS FOR THE TREATMENT OF
ALZHEIMER'S DISEASE

FIELD OF THE INVENTION

5 This invention relates to novel compositions and methods for the treatment of Alzheimer's disease and other central and peripheral nervous system disorders involving cholinergic hypofunction.

SUMMARY OF THE RELATED ART

10 Cognitive disorders, including Alzheimer's disease, are generally accompanied by symptoms of forgetfulness, confusion, memory loss and other symptoms resulting from aging, brain injury, or disease. The concomitant decrease in cognitive function during the aging process has been documented in various mammals, including humans. In particular, presenile and senile primary
15 degenerative dementia appear to be common causes of mental deterioration among the elderly. The symptoms of cognitive disorder appear to be associated with decreased acetylcholine synthesis as well as impairment of the ACh receptive neurons. The activity of the enzyme choline acetyltransferase (ChAT), which catalyzes the synthesis of acetylcholine from choline and acetyl coenzyme A, can be severely reduced as reflected by the loss of cholinergic (acetylcholine releasing)
20 nerve endings in the hippocampus. Conversely, post-synaptic muscarinic receptors are generally intact. The cholinergic terminals are thus recognized as critically important to memory function.

 Alzheimer's disease is a chronic neurodegenerative disorder. With increased life expectancy, the number of Alzheimer's patients can be predicted to
25 rise rapidly in the future. Decline in cognitive function is the cardinal symptom of Alzheimer's disease, and a beneficial treatment effect on cognitive function is

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today a *sine qua non* of efficacy for an antidementia drug. Acetylcholine-synthesizing neurons in the basal forebrain region and their cortical synaptic connections exhibit a well-characterized degeneration in Alzheimer's disease. Thus, most drug research targeting Alzheimer's disease has been focused on providing symptomatic cognitive enhancement by increasing acetylcholine transmission.

Deficits in sustained attention are consistently reported in aged and demented humans (Alexander DA. Psychol. Rep., 1973;32:229-230.) The role of an attentional deficit and the cholinergic contribution to these deficits may be important in Alzheimer's disease. These deficits in attention are similar to those produced by scopolamine in otherwise cognitively intact humans (Wesnes and Warburton, Neuropsychobiology, 1983; 9:154-157; Wesnes and Warburton, Drug Dev. Res., 1988,12:279-286). Scopolamine has been used in healthy volunteers as a model of dementia to screen for compounds for potential therapeutic usefulness (Wesnes, et al., Ann. NY Acad. Sci., 1991;640:268-271).

Presently, there are three general approaches to enhance cholinergic transmission in the central nervous system. The first approach is to enhance cholinergic neurons by excessive exposure to a form of choline. Such attempts have been mildly successful, but only in the early stages of Alzheimer's disease. The second approach involves postsynaptic direct stimulation of muscarinic receptors by oral or intravenous administration of drugs. This approach is limited because of a low therapeutic index (a high effective dose to toxic dose ratio). In addition, a variety of undesirable, nonspecific side effects would be expected. The third approach involves the inhibition of acetylcholinesterase, the enzyme that metabolizes acetylcholine. At present, Alzheimer's disease is treated by 1,2,3,4-tetrahydro-5-aminoacridine (Tacrine) (US Patent No. 4,816,456), a therapy involving inhibition of acetylcholinesterase activity. The most frequent adverse events established during clinical trials of high-dose 1,2,3,4-tetrahydro-5-aminoacridine were elevated liver transaminase levels, followed by dose-related peripheral cholinergic effects primarily affecting the gastrointestinal tract (nausea, vomiting, diarrhea, dyspepsia, anorexia). The risks of 1,2,3,4-tetrahydro-5-aminoacridine treatment are small relative to its benefits, but achieving optimal

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1,2,3,4-tetrahydro-5-aminoacridine treatment response requires careful dose-titration and transaminase monitoring. Accordingly, new compositions and methods for treating diseases resulting from cholinergic hypofunction are desired.

SUMMARY OF INVENTION

5 The present invention comprises new compositions and methods for treating central and peripheral nervous system disorder involving cholinergic hypofunction. The compositions of the present invention comprise a combination of acetylcholinesterase inhibitors and muscarinic agonists. The methods of the invention comprise the use of these compositions for the treatment of cholinergic
10 hypofunction.

 This invention is an improvement over previous compositions and treatment methods because the two agents can be safely and effectively administered in combination using doses of each agent that by themselves showed no activity. Positive results with minimal side effects are obtained with the present
15 composition. The active dose range for the compounds is widened in the present combination. The combined treatment may eliminate the need to individually titrate doses and also increase the separation between efficacy and adverse events.

 A preferred embodiment utilizes an acetylcholinesterase inhibitor selected from tacrine or donepezil (E2020) and a muscarinic agonist selected from
20 milameline (CI-979), xanomeline (LY246708), or CI-1017.

BRIEF DESCRIPTION OF THE DRAWINGS

 A more complete appreciation of the invention and many of the attendant advantages thereof will more readily be understood by reference to the following detailed description when considered in connection with the accompanying
25 drawings, wherein:

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Figure 1 shows reversal of scopolamine-induced impairment of continuous performance in rhesus monkeys by administration of 1,2,3,4-tetrahydro-5-aminoacridine (tacrine).

5 Figure 2 shows reversal of scopolamine-induced impairment of continuous performance in rhesus monkeys by administration of 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine (E2020).

Figure 3 shows reversal of scopolamine-induced impairment of continuous performance in rhesus monkeys by administration of 1,2,5,6-tetrahydro-1-methyl-3-pyridinecarboxaldehyde-O-methyl-oxime (milameline, CI-979).

10 Figure 4 shows reversal of scopolamine-induced impairment of continuous performance in rhesus monkeys by administration, in combination, of tacrine and CI-979.

Figure 5 shows reversal of scopolamine-induced impairment of continuous performance in rhesus monkeys by administration, in combination, of 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine (E2020) and CI-979 (milameline).

Figure 6 shows the observable side effects seen upon a 1,2,3,4-tetrahydro-5-aminoacridine-produced decrease in responses on continuous performance tasks in rhesus monkeys.

20 Figure 7 shows a dose-response decrease in the absence of any observable side effects in responses on a continuous performance task in rhesus monkeys upon administration of 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine.

Figure 8 shows a dose-response decrease in responses on a continuous performance task in rhesus monkeys upon administration of CI-979.

25 Figure 9 shows a dose-response decrease in responses on a continuous performance task in rhesus monkeys upon administration, in combination, of tacrine and CI-979 (milameline).

Figure 10 shows a dose-response decrease in responses on a continuous performance task in rhesus monkeys upon administration, in combination, of 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine (E2020) and milameline.

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DETAILED DESCRIPTION OF THE INVENTION

We have surprisingly discovered that combining an acetylcholinesterase inhibitor and a muscarinic agonist broadened the dose-response effect by significantly reversing a scopolamine-induced behavioral impairment. These results suggest that the combination of acetylcholinesterase inhibitors and muscarinic agonists is useful in the treatment of cognitive dysfunction arising from cholinergic hypofunction, such as Alzheimer's disease. The improved performance with the combination treatment occurred at lower doses than required for either compound alone. This effect at lower doses when given in combination provides a greater separation between the efficacious dose and the dose producing peripheral side effects. No difference in drug plasma levels was observed for either agent when the acetylcholinesterase inhibitor 1,2,3,4-tetrahydro-5-aminoacridine (tacrine) (U.S. Patent No. 4,816,456) and the muscarinic agonist 1,2,5,6-tetrahydro-1-methyl-3-pyridinecarboxaldehyde-O-methyloxime (milameline CI-979) (U.S. Patent No. 5,219,872) were used in combination as compared to when used alone (see Table 1). These results establish that the combined treatment may eliminate the need to individually titrate doses and also increase the separation between efficacy and adverse events.

TABLE 1. Plasma Levels of 1,2,5,6-Tetrahydro-1-methyl-3-pyridinecarboxaldehyde-O-methyloxime and 1,2,3,4-Tetrahydro-5-aminoacridine Alone and in Combination in Adult Rhesus Monkeys

Drug	Dose, IM (mg/kg)	Plasma levels (ng/mL)	Plasma levels (ng/mL) In combination ^a
1,2,5,6-tetrahydropyridin-3-carboxaldehyde oxime	0.003	1.12 (1.27-0.97)	1.35 (1.42-1.28)
1,2,3,4-tetrahydro-5-aminoacridine	0.32	34.4 (41.7-30.3)	29.1 (33.7-24.5)

^a Combination: 1,2,5,6-Tetrahydro-1-methyl-3-pyridinecarboxaldehyde-O-methyl-oxime (0.003 mg/kg) + 1,2,3,4-tetrahydro-5-aminoacridine (0.32 mg/kg)
n = 2 except 1,2,3,4-tetrahydro-5-aminoacridine alone group was n = 4.

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In accordance with the foregoing, the present invention comprises a composition of matter comprising the combination of at least one acetylcholinesterase inhibitor and at least one muscarinic agonist. Such acetylcholinesterase inhibitors are well-known in the art and include but are not limited to physostigmine (eserine), monoamine acridines and their derivatives (U.S. Patent No. 4,816,456), 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine (Aricept, donepezil, E2020) (U.S. Patent No. 4,895,841), piperidine and piperazine derivatives (U.S. Patent No. 5,041,455), piperidinyl-alkanoyl heterocyclic compounds (EP 487071), N-benzyl-piperidine derivatives (U.S. Patent No. 5,106,856), 4-(1-benzyl:piperidyl)-substituted fused quinoline derivatives (EP 481429-A) and cyclic amide derivatives (EP 468187). Other typical acetylcholinesterase inhibitors include carbonic acid derivatives such as those described in U.S. Patent No. 5,602,176 (e.g. exelon, ENA-713, which is (S)-[N-ethyl-3-[(1-dimethylamino)ethyl]-N methyl-phenyl-carbamate]), and phosphonate compounds such as O, O-dimethyl-(1-hydroxy-2, 2,2-trichloroethyl) phosphonate (metrifonate, or trichlofon). Benzazepinols such as galantamine are also useful acetylcholinesterase inhibitors.

Similarly, muscarinic agonists are well-known in the art and include but are not limited to arecoline, tetrahydropyridine derivatives such as 1,2,5,6-tetrahydropyridin-3-carboxaldehyde oxime, and 1,2,5,6-tetrahydro-1-methyl-3-pyridinecarboxaldehyde-O-methyloxime hydrochloride (milameline, CI-979, U.S. Patent No. 5,219,872). Other pyridine derivatives include 3-(3-hexyloxy-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine (xanomeline, LY246708, U.S. Patent No. 5,043,345), and 3-(3-hexylthio-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine U.S. Patent No. 5,041,455). Additional muscarinic agonists include talsaclidine, which is (3R)-3-(2-propynyloxy)quinclidine, and memric, which is R-(Z)- α -(methoxyimins)-1-azobicyclo [2.2.2] octane-3-acetonitrile, and YM-796, which is (S)-(-)-2,8-dimethyl-3-methylene-1-oxa-8-azaspiro-[4.5]decane L-tartrate monohydrate, (U.S. 4,996,210).

Certain pyridine-like derivatives are aza-bicyclics such as 1-aza-bicyclo [2.2.1]heptan-3-one-O-[3-(3-methoxyphenyl)-prop-2-ynyl]oxime (CI-1017, U.S.

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Patent No. 5,346,911), and 3-(3-butylthio-1,2,5-thiadiazol-4-yl)-1-azabicyclo [2.2.2]-octane. Preferably, the composition comprises one acetylcholinesterase inhibitor and one muscarinic agonist.

5 In a preferred embodiment, the acetylcholinesterase inhibitor comprises 1,2,3,4-tetrahydro-5-aminoacridine or 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine, and the muscarinic agonist comprises 1,2,5,6-tetrahydro-1-methyl-3-pyridinecarboxaldehyde-O-methyloxime or 1-aza-bicyclo[2.2.1]heptan-3-one-O-[3-(3-methoxyphenyl)-prop-2-ynyl]oxime.

10 As used herein, "acetylcholinesterase inhibitor" and "muscarinic agonist" include their respective pharmaceutically acceptable salts, such as hydrochlorides, tartrates, and the like.

All of the foregoing patents are incorporated herein by reference for their disclosure of typical acetylcholinesterase inhibitors and muscarinic agonists.

15 In a preferred embodiment, the invention comprises a composition of matter wherein the ratio of acetylcholinesterase inhibitor relative to muscarinic agonist is between 0.5:1 and 1000:1 by weight. In a more preferred embodiment, the ratio of acetylcholinesterase inhibitor relative to muscarinic agonist is between 1:1 and 500:1 by weight. In a most preferred embodiment, the ratio is between 3:1 and 350:1 by weight.

20 Aside from their utility for treating cholinergic deficit states, the compositions of the invention are useful research tools for studying the physiology of such states.

In another aspect, the present invention comprises a method of treating central and peripheral nervous system disorders involving cholinergic
25 hypofunction in a mammal, said method comprising administering to a mammal an amount of at least one acetylcholinesterase inhibitor and at least one muscarinic agonist effective in treating the cholinergic hypofunction. As used herein, central and peripheral nervous system disorders involving cholinergic hypofunction include but are not limited to dementias, amnesias, cerebral insufficiencies, and
30 psychiatric disturbances in the central nervous system and neuronal and smooth muscle dysfunction of the gut, bladder, and secretory glands in the peripheral nervous system. The acetylcholinesterase inhibitor(s) and muscarinic agonists(s)

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can be administered together as a composition, or may be administered separately. If administered separately, such subsequent administration should occur within a time period such that the drugs can act in concert within the body. In a preferred embodiment of the instant invention, the acetylcholinesterase inhibitor comprises
5 1,2,3,4-tetrahydro-5-aminoacridine or 1-benzyl-4-(5,6-dimethoxy-1-indanon)-
2-yl)methylpiperidine and the muscarinic agonist comprises CI-979, CI-1017, or xanomeline.

The present invention is further directed to the method of treating central and peripheral nervous system disorders involving cholinergic hypofunction in a
10 mammal, the method comprising the administration to a mammal of a ratio of
acetylcholinesterase inhibitor relative to muscarinic agonist of between 0.5:1 and
1000:1 by weight. In a more preferred embodiment, the ratio of acetyl-
cholinesterase inhibitor relative to muscarinic agonist is between 1:1 and 500:1 by
weight. In a most preferred embodiment, the ratio is between 3:1 and 350:1 by
15 weight. The combinations are synergistic for treating Alzheimer's disease.

The compositions of the invention can be administered using art-recognized techniques. Preferably, the acetylcholinesterase inhibitor and the muscarinic agonist are administered orally, transdermally, or parenterally. In
20 general, however, the compositions of the invention can be administered using the
same art-recognized techniques used for administration of acetylcholinesterase
inhibitors and muscarinic agonists. Accordingly, techniques of administration
need not be repeated here.

In another aspect, the invention provides pharmaceutical compositions comprising a composition according to the invention and a pharmaceutically
25 acceptable carrier or diluent and optionally other adjuvants. Acceptable carriers,
diluent, and adjuvants are any of those commercially used in the art, in particular,
those used in pharmaceutical compositions of acetylcholinesterase inhibitors and
muscarinic agonists. Accordingly, such carriers, diluents, and adjuvants need not
be repeated here.

30 The following examples illustrate the present invention and will enable
others skilled in the art to understand the invention more completely.

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EXAMPLE 1

Reversal of scopolamine-induced decreases in cognitive function in rhesus monkeys by the administration of acetylcholinesterase inhibitors and a muscarinic agonist both alone and in combination

5 Scopolamine has been used in healthy volunteers as a model of dementia to screen for compounds for potential therapeutic usefulness in the treatment of cognitive disorders (Wesnes K, et al., Ann. NY Acad. Sci., 1991;640:268-271). We therefore introduced scopolamine to impair the performance of rhesus monkeys on a continuous performance task. Diminution of response was seen
10 without a concomitant decrease in response accuracy, suggesting that scopolamine impaired the ability of these monkeys to remain attentive across time (vigilance). Similar effects have been demonstrated in both rat and human (Brown and Warburton, Psychol. Sci., 1971, 22:297-298; Parrott Psychopharmacol. 1986;89:347-354; Wesnes K. and Warburton D.M., Neuropsychobiology 1983,9:154-157). In monkeys, this impaired performance was shown to be
15 mediated through blockade of central muscarinic receptors by directly infusing scopolamine into the lateral ventricle (Callahan et al., Neurobiol. Aging. 1993;14:147-151). These changes with scopolamine are thought to result from a decrease in central cholinergic function that interferes with the efficient selection
20 and processing of information. Scopolamine, given to healthy volunteers, has been reported to produce peripheral effects that begin to subside within 60 minutes after dosing, while cognitive performance remains impaired out to 2 hours on tasks measuring vigilance and continuous performance. To minimize peripheral effects with intramuscular dosing in monkeys, scopolamine was administered 90 minutes
25 before testing.

 The subjects were 6 adult rhesus monkeys 12+ years old, 4 females and 2 males. Continuous performance was measured using a microcomputer-controlled test environment. Briefly, test-experienced rhesus monkeys were presented a stimulus consisting of a yellow square randomly displayed on a color
30 television monitor. Stimulus duration (1, 2, or 4 seconds) and intertrial interval (1, 2, or 3 seconds) were randomly determined in a complete block design. Animals

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were rewarded for responding to a target object displayed on the screen of a touch-sensitive color television monitor (CRT) by delivery of a 190 mg banana-flavored food pellet and presentation of a tone (ascending series of tones; 500 msec duration). Inaccurate responses were signaled by tone (700 Hz, 500 msec) but were not rewarded. Display of the target object on the CRT was randomized with respect to time and spatial location. Test sessions consisted of 150 trials. Responses were expressed as means \pm standard error of the mean. Differences between the means were analyzed using t-test for paired observations and were considered significant at a level of $p < 0.05$.

Scopolamine (0.003 or 0.006 mg/kg) was injected intramuscularly (IM) 90 minutes before testing. In these studies, 3 of the 6 monkeys were unimpaired by 0.003 mg/kg and required 0.006 mg/kg of scopolamine for testing. In the first set of experiments, 1,2,3,4-tetrahydro-5-aminoacridine (0.03, 0.10, 0.32, and 1.0 mg/kg), 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine (0.03, 0.10, 0.32, and 1.0 mg/kg), 1,2,5,6-tetrahydro-1-methyl-3-pyridine-carboxaldehyde-O-methyloxime (0.001, 0.003, and 0.010 mg/kg), or a combination of 1,2,3,4-tetrahydro-5-aminoacridine and 1,2,5,6-tetrahydro-1-methyl-3-pyridinecarboxaldehyde-O-methyloxime or 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine, and 1,2,5,6-tetrahydro-1-methyl-3-pyridine-carboxaldehyde-O-methyloxime were given IM 30 minutes before testing and 60 minutes after an impairing dose of scopolamine. All compounds were dissolved in 0.9% saline for injection and all doses refer to free base.

Scopolamine significantly decreased the number of correct responses made by these monkeys. Figures 1 and 2 show that this impaired performance produced by scopolamine was significantly reversed by 0.32 mg/kg of 1,2,3,4-tetrahydro-5-aminoacridine (Figure 1) or by 0.10 mg/kg of 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine (Figure 2) when either compound was administered 60 minutes after scopolamine and 30 minutes before testing.

Figure 3 shows that CI-979, 1,2,5,6-tetrahydro-1-methyl-3-pyridine-carboxaldehyde-O-methyloxime similarly produced an improvement in performance that was statistically significant only when using the best dose effect

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of CI-979 for each monkey, to account for individual variability. This best dose of CI-979 (0.003 or 0.010 mg/kg) in each monkey correlated with the maximum-tolerated dose free of side effects. Figures 4 and 5 show that combined treatment with 1,2,3,4-tetrahydro-5-aminoacridine (0.1 and 0.32 mg/kg) and CI-979 (0.001 and 0.003 mg/kg) (Figure 4) or with 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine (0.03 and 0.10 mg/kg) and CI-979 (0.001 and 0.003 mg/kg) (Figure 5) significantly improved performance at each of the dose combinations tested. Furthermore, at low doses 1,2,3,4-tetrahydro-5-aminoacridine (0.1 mg/kg) and CI-979 (0.001 and 0.003 mg/kg), or 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine (0.032 mg/kg) and CI-979 (0.001 mg/kg), given in combination reversed the scopolamine-induced impairment to a greater extent than did equivalent doses of either compound alone.

These effects are similar to those reported in mice, where a potentiation of cognitive function by various combinations of the cholinergics arecoline, edrophonium and oxotremorine was observed (Flood, et al., *Neurobiol. Aging*, 1983;4:37-43). These results in mice were obtained after direct injection of the combinations into the brain, thus minimizing the impact of potential peripheral nervous system side effects. In contrast, the instant invention utilizes intramuscular injections, which may activate both central and peripheral nervous system side effects.

EXAMPLE 2

Administration of acetylcholinesterase inhibitors and a muscarinic agonist both alone and in combination to rhesus monkeys in the absence of scopolamine

In the second set of experiments, 1,2,3,4-tetrahydro-5-aminoacridine (0.10, 0.32, and 1.0 mg/kg), 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine (0.03, 0.10, and 0.32 mg/kg) and CI-979 (0.001, 0.003, and 0.010 mg/kg), or a combination of 1,2,3,4-tetrahydro-5-aminoacridine and CI-979 or 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine and CI-979 were given IM 30 minutes before testing, in the absence of scopolamine. All compounds were dissolved in 0.9% saline for injection and all doses refer to free

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base. Figures 6 and 7 show that 1,2,3,4-tetrahydro-5-aminoacridine (1.0 mg/kg) (Figure 6) or 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine (0.32 mg/kg) (Figure 7) given alone 30 minutes before testing significantly decreased responses. In both cases, the impaired behavioral performance was observed in the absence of any observable side effects. Figure 8 shows that CI-979 given alone similarly produced a decrease in responses in 3 of 6 monkeys at 0.010 mg/kg that was not statistically significant. Excessive peripheral cholinergic stimulation (salivation, emesis) was observed in 2 of these monkeys. Figure 9 shows that combined treatment with 1,2,3,4-tetrahydro-5-aminoacridine (0.32 mg/kg) and CI-979 (0.001 and 0.003 mg/kg), in the absence of scopolamine, did not produce adverse events significantly different from those seen with either compound alone, and no obvious peripheral side effects were observed. Figure 10 similarly shows that combined treatment with 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine (0.03 and 0.10 mg/kg) and CI-979 (0.001 and 0.003 mg/kg) in the absence of scopolamine produced no obvious peripheral side effects or significant changes in the number of responses.

Thus, in neither case did the combination treatment potentiate the adverse effects of the individual compounds. Further, the combinations did not produce any adverse effects at the combination doses tested, which significantly reversed scopolamine-induced behavioral impairments, but were below the doses at which either compound produced side effects when given alone.

In each of these treatments the decrease in responding occurred across all stimulus durations. Sedation or motor impairments produce a selective decrease at short relative to long stimulus durations. This did not occur in the present study, suggesting that the disruptive effects observed were not due to general cognitive dulling but were probably due to peripheral side effects.

It is to be understood that the invention is not to be limited to the exact details of operation, or to the exact compounds, compositions, methods, procedures, or embodiments shown and described, as obvious modifications and equivalents will be apparent to one skilled in the art, and the invention is therefore to be limited only by the full scope of the appended claims.

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CLAIMS

What is claimed is:

1. A composition comprising an effective amount of an acetylcholinesterase inhibitor in combination with an effective amount of a muscarinic agonist.
- 5 2. A combination of Claim 1 wherein the acetylcholinesterase inhibitor is tacrine, donepezil, exelon, metrifonate, or galantamine.
3. A combination of Claim 2 wherein the muscarinic agonist is milameline, xanomeline, 1-aza-bicyclo[2.2.1]heptan-3-one-O-[3-(3-methoxy-phenyl)-prop-2-ynyl]-oxime, talsaclidine, YM-796, or memric.
- 10 4. A composition of matter comprising the combination of 1,2,3,4-tetrahydro-5-aminoacridine and 1,2,5,6-tetrahydro-1-methyl-3-pyridine-carboxaldehyde-O-methyloxime .
5. A composition of matter comprising the combination of 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine and
15 1,2,5,6-tetrahydro-1-methyl-3-pyridinecarboxaldehyde-O-methyloxime
6. The composition of Claim 4 wherein the ratio of said 1,2,3,4-tetrahydro-5-aminoacridine relative to said 1,2,5,6-tetrahydro-1-methyl-3-pyridine-carboxaldehyde-O-methyloxime is between 0.5:1 and 1000:1 by weight.
7. The composition of Claim 4 wherein the ratio of said 1,2,3,4-tetrahydro-
20 5-aminoacridine relative to said 1,2,5,6-tetrahydro-1-methyl-3-pyridine-carboxaldehyde-O-methyloxime is between 1:1 and 500:1 by weight.
8. The composition of Claim 4 wherein the ratio of said 1,2,3,4-tetrahydro-5-aminoacridine relative to said 1,2,5,6-tetrahydro-1-methyl-3-pyridine-carboxaldehyde-O-methyloxime is between 3:1 and 350:1 by weight.

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9. The composition of Claim 5, wherein the ratio of said 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine relative to said 1,2,5,6-tetrahydro-1-methyl-3-pyridinecarboxaldehyde-O-methyloxime is between 0.5:1 and 1000:1 by weight.
- 5 10. The composition of Claim 5, wherein the ratio of said 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine relative to said 1,2,5,6-tetrahydro-1-methyl-3-pyridinecarboxaldehyde-O-methyloxime is between 1:1 and 500:1 by weight.
- 10 11. The composition of Claim 5, wherein the ratio of said 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine relative to said 1,2,5,6-tetrahydro-1-methyl-3-pyridinecarboxaldehyde-O-methyloxime is between 3:1 and 350:1 by weight.
12. A pharmaceutical composition comprising a composition according to Claim 1 and pharmaceutically acceptable carrier.
- 15 13. A pharmaceutical composition comprising a composition according to Claim 2 and pharmaceutically acceptable carrier.
14. A pharmaceutical composition comprising a composition according to Claim 3 and pharmaceutically acceptable carrier.
- 20 15. A pharmaceutical composition comprising a composition according to Claim 4 and pharmaceutically acceptable carrier.
16. A pharmaceutical composition comprising a composition according to Claim 5 and pharmaceutically acceptable carrier.
17. A pharmaceutical composition comprising a composition according to Claim 6 and pharmaceutically acceptable carrier.

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18. A pharmaceutical composition comprising a composition according to Claim 7 and pharmaceutically acceptable carrier.
19. A pharmaceutical composition comprising a composition according to Claim 8 and pharmaceutically acceptable carrier.
- 5 20. A method of treating central and peripheral nervous system disorders involving cholinergic hypofunction in a mammal, said method comprising administering to said mammal a cholinergic deficit state-reducing amount of at least one acetylcholinesterase inhibitor and at least one muscarinic agonist.
- 10 21. The method of Claim 20 wherein the ratio of said acetylcholinesterase inhibitor relative to said muscarinic agonist is between 0.5:1 and 1000:1 by weight.
22. The method of Claim 20 wherein the ratio of said acetylcholinesterase inhibitor relative to said muscarinic agonist is between 1:1 and 500:1 by weight.
- 15 23. The method of Claim 20 wherein the ratio of said acetylcholinesterase inhibitor relative to said muscarinic agonist is between 3:1 and 350:1 by weight.
- 20 24. A method of treating central and peripheral nervous system disorders involving cholinergic hypofunction in a mammal, said method comprising administering to said mammal a cholinergic deficit state-reducing amount of a pharmaceutical composition according to Claim 12.
- 25 25. A method of treating central and peripheral nervous system disorders involving cholinergic hypofunction in a mammal, said method comprising administering to said mammal a cholinergic deficit state-reducing amount of a pharmaceutical composition according to Claim 13.

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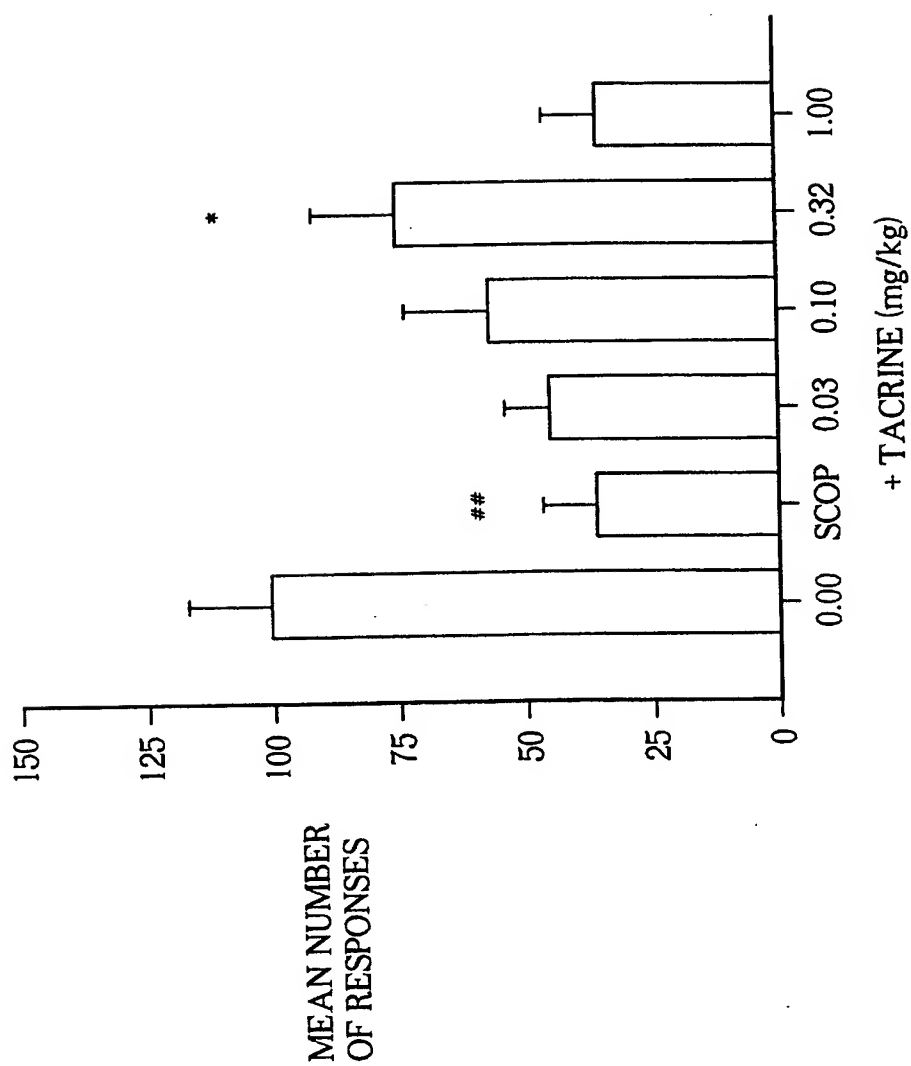
26. A method of treating central and peripheral nervous system disorders involving cholinergic hypofunction in a mammal, said method comprising administering to said mammal a cholinergic deficit state-reducing amount of a pharmaceutical composition according to Claim 14.
- 5 27. A method of treating central and peripheral nervous system disorders involving cholinergic hypofunction in a mammal, said method comprising administering to said mammal a cholinergic deficit state-reducing amount of a pharmaceutical composition according to Claim 15.
- 10 28. A method of treating central and peripheral nervous system disorders involving cholinergic hypofunction in a mammal, said method comprising administering to said mammal a cholinergic deficit state-reducing amount of a pharmaceutical composition according to Claim 16.
- 15 29. A method of treating central and peripheral nervous system disorders involving cholinergic hypofunction in a mammal, said method comprising administering to said mammal a cholinergic deficit state-reducing amount of a pharmaceutical composition according to Claim 17.
- 20 30. A method of treating central and peripheral nervous system disorders involving cholinergic hypofunction in a mammal, said method comprising administering to said mammal a cholinergic deficit state-reducing amount of a pharmaceutical composition according to Claim 18.
31. A method of treating central and peripheral nervous system disorders involving cholinergic hypofunction in a mammal, said method comprising administering to said mammal a cholinergic deficit state-reducing amount of a pharmaceutical composition according to Claim 19.
- 25 32. The method of Claim 24 wherein said central and peripheral nervous system disorder is Alzheimer's disease.

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33. The method of Claim 25, wherein said central and peripheral nervous system disorder is Alzheimer's disease.
34. The method of Claim 26, wherein said central and peripheral nervous system disorder is Alzheimer's disease.
- 5 35. The method of Claim 27, wherein said central and peripheral nervous system disorder is Alzheimer's disease.
36. The method of Claim 28, wherein said central and peripheral nervous system disorder is Alzheimer's disease.
- 10 37. The method of Claim 29, wherein said central and peripheral nervous system disorder is Alzheimer's disease.
38. The method of Claim 30, wherein said central and peripheral nervous system disorder is a Alzheimer's disease.
39. The method of Claim 31, wherein said central and peripheral nervous system disorder is Alzheimer's disease.

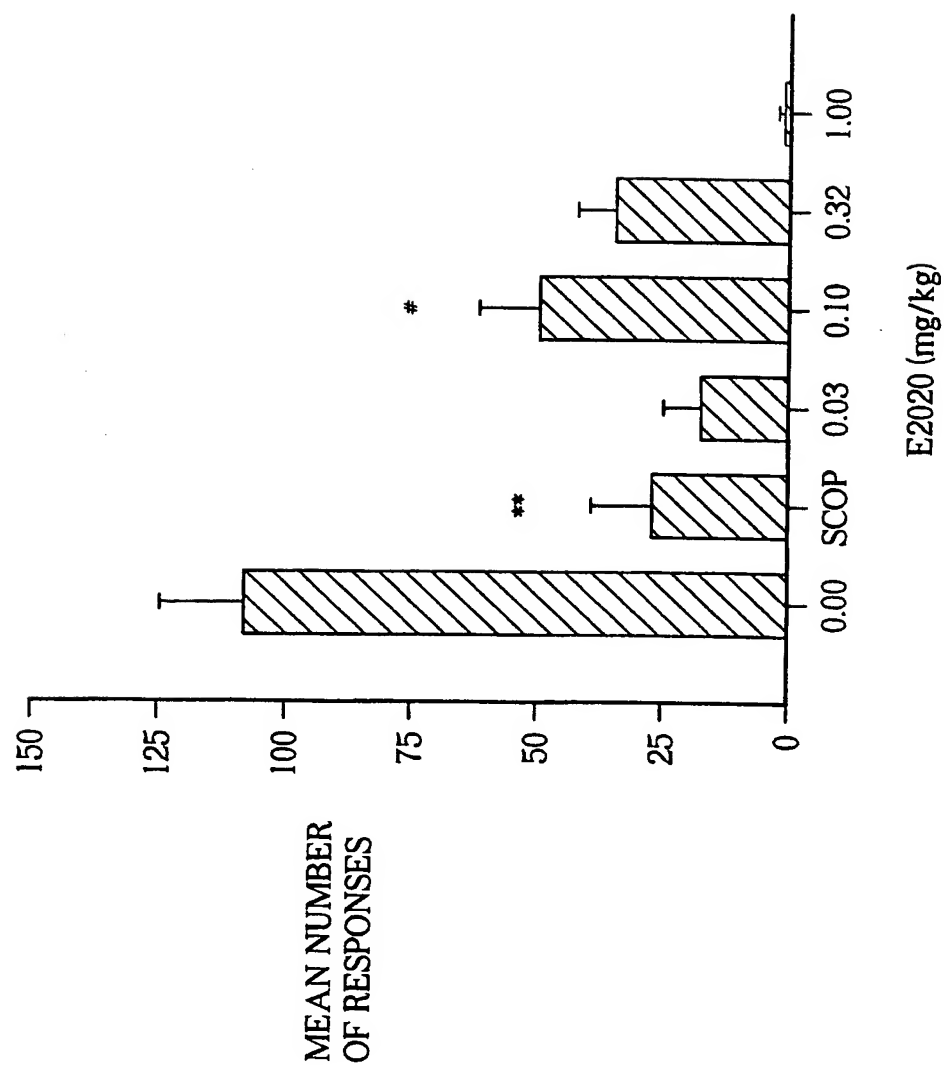
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FIG-1 TACRINE REVERSED THE SCOPOLAMINE-INDUCED IMPAIRMENT OF CONTINUOUS PERFORMANCE IN RHESUS MONKEYS (N = 6/dose)

**SUBSTITUTE SHEET (RULE 26)**

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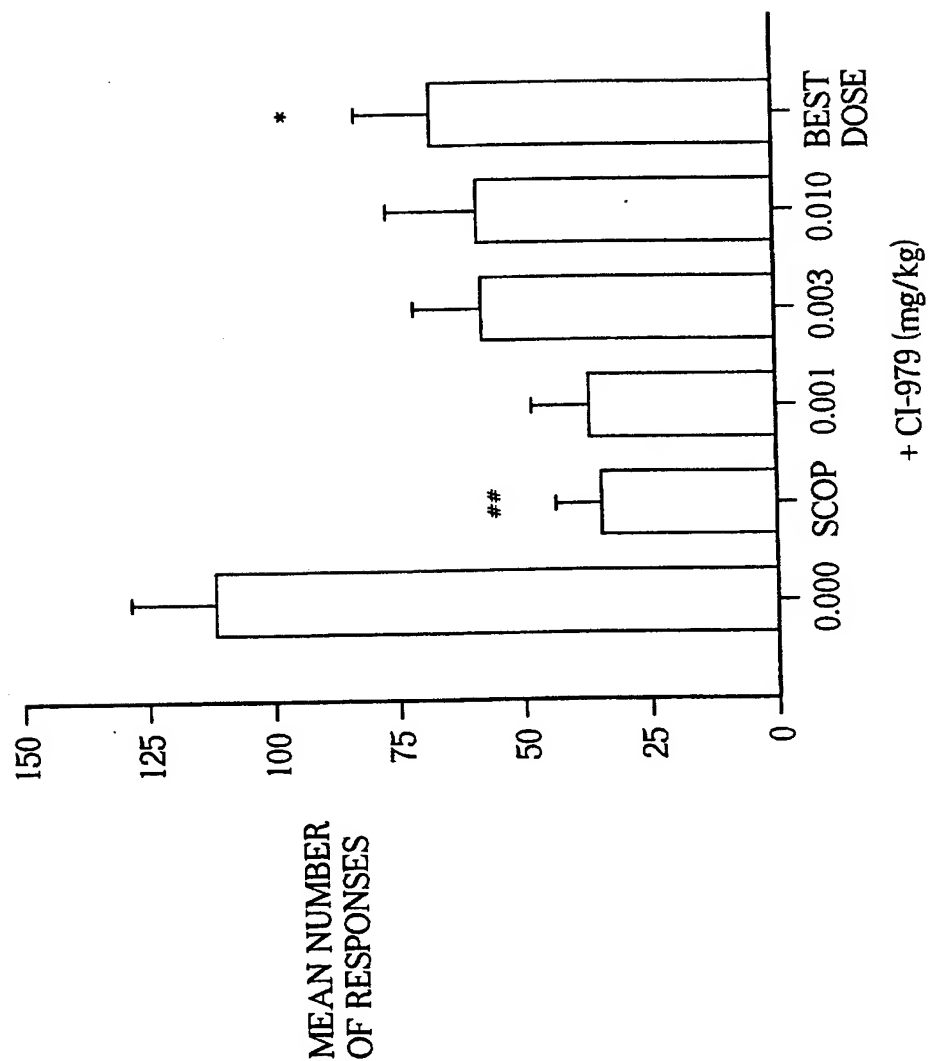
FIG-2



SUBSTITUTE SHEET (RULE 26)

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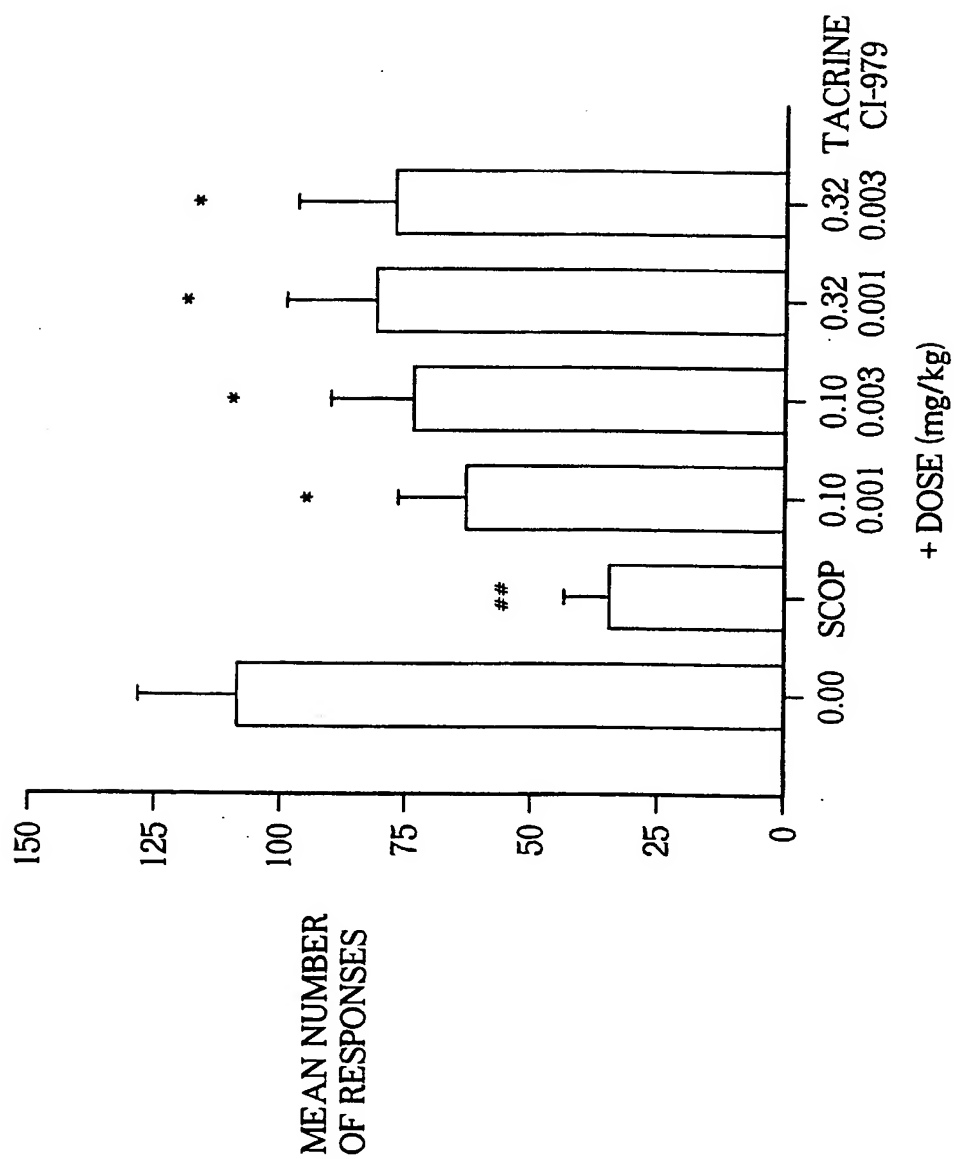
FIG-3 CI-979 REVERSED THE SCOPOLAMINE-INDUCED IMPAIRMENT OF CONTINUOUS PERFORMANCE IN RHESUS MONKEYS (N = 6/dose)



SUBSTITUTE SHEET (RULE 26)

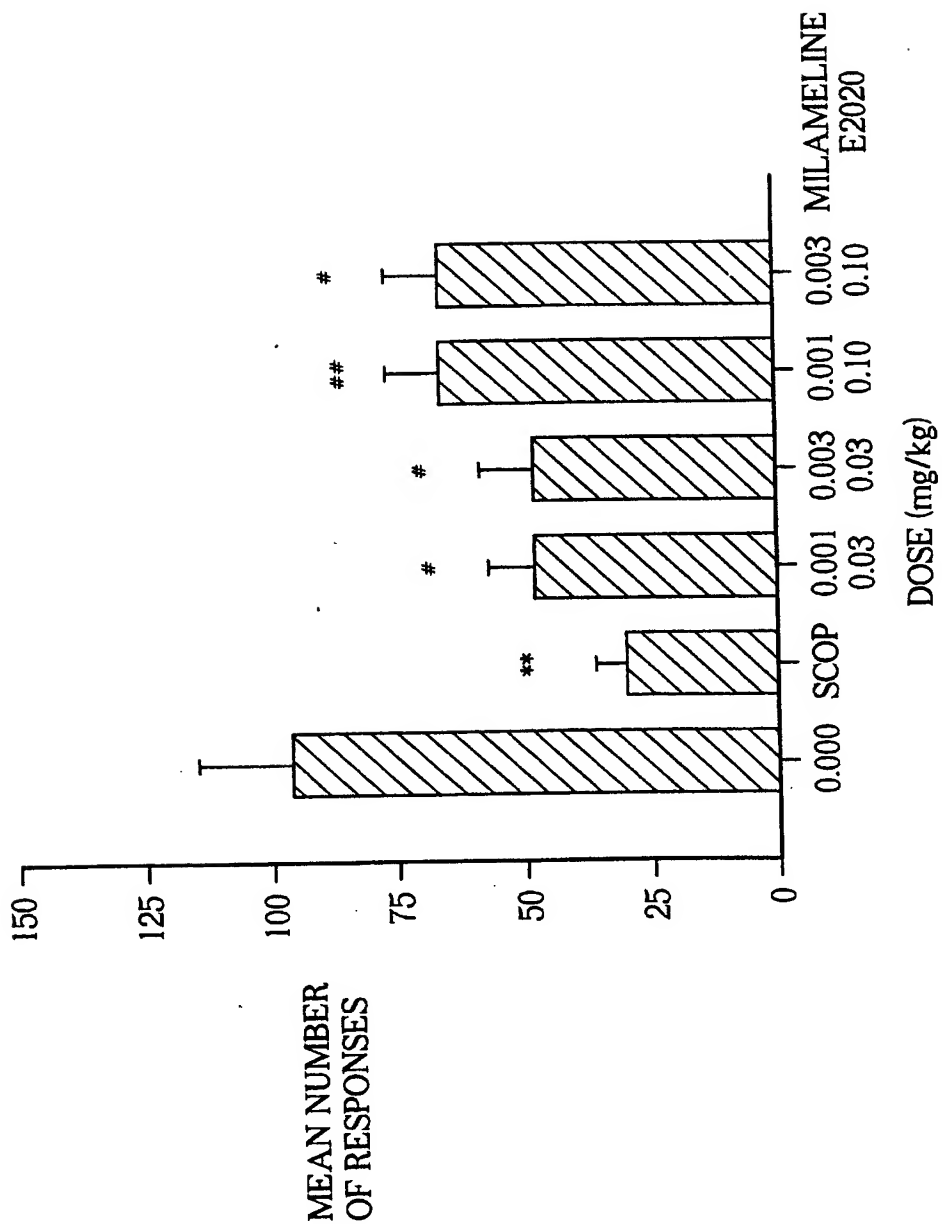
4/10

FIG-4 TACRINE AND CI-979 GIVEN IN COMBINATION REVERSED THE SCOPOLAMINE-INDUCED IMPAIRMENT OF CONTINUOUS PERFORMANCE IN RHESUS MONKEYS (N = 6/dose)



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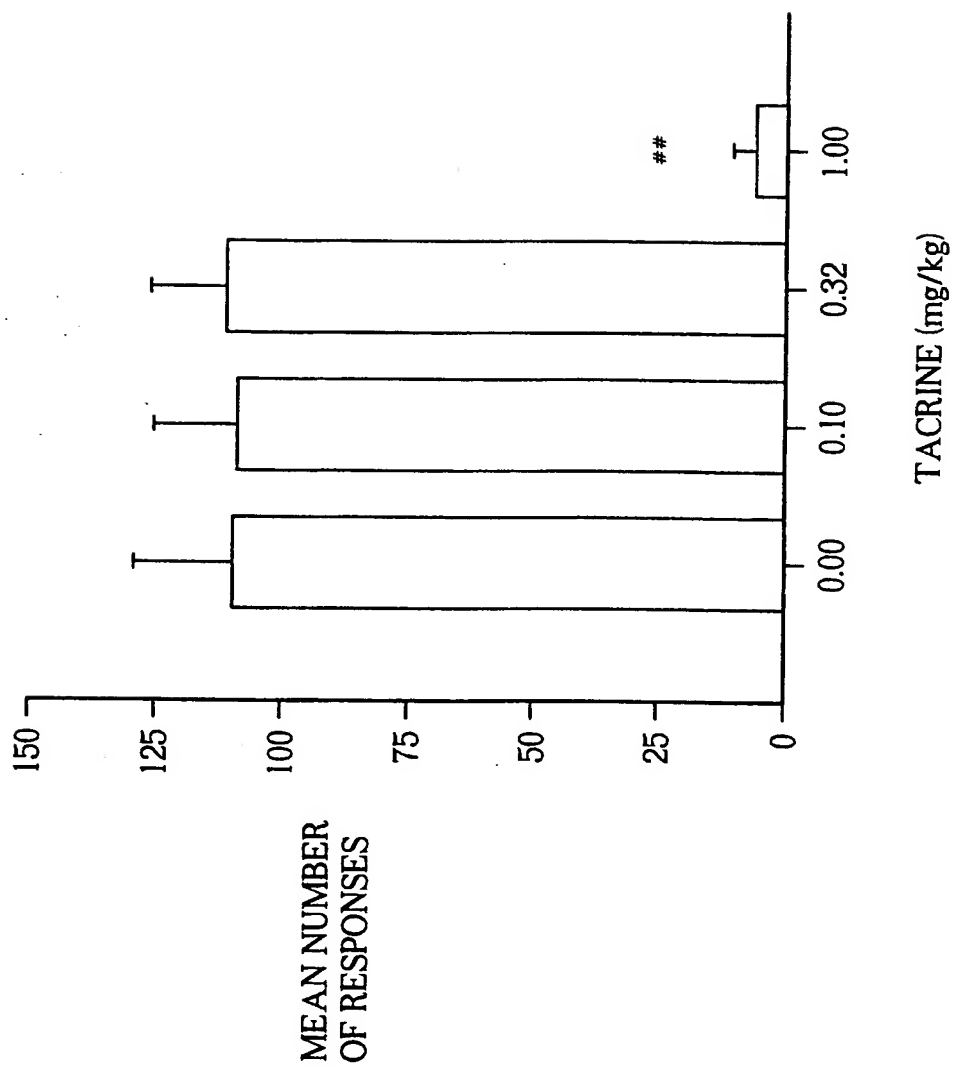
FIG-5



SUBSTITUTE SHEET (RULE 26)

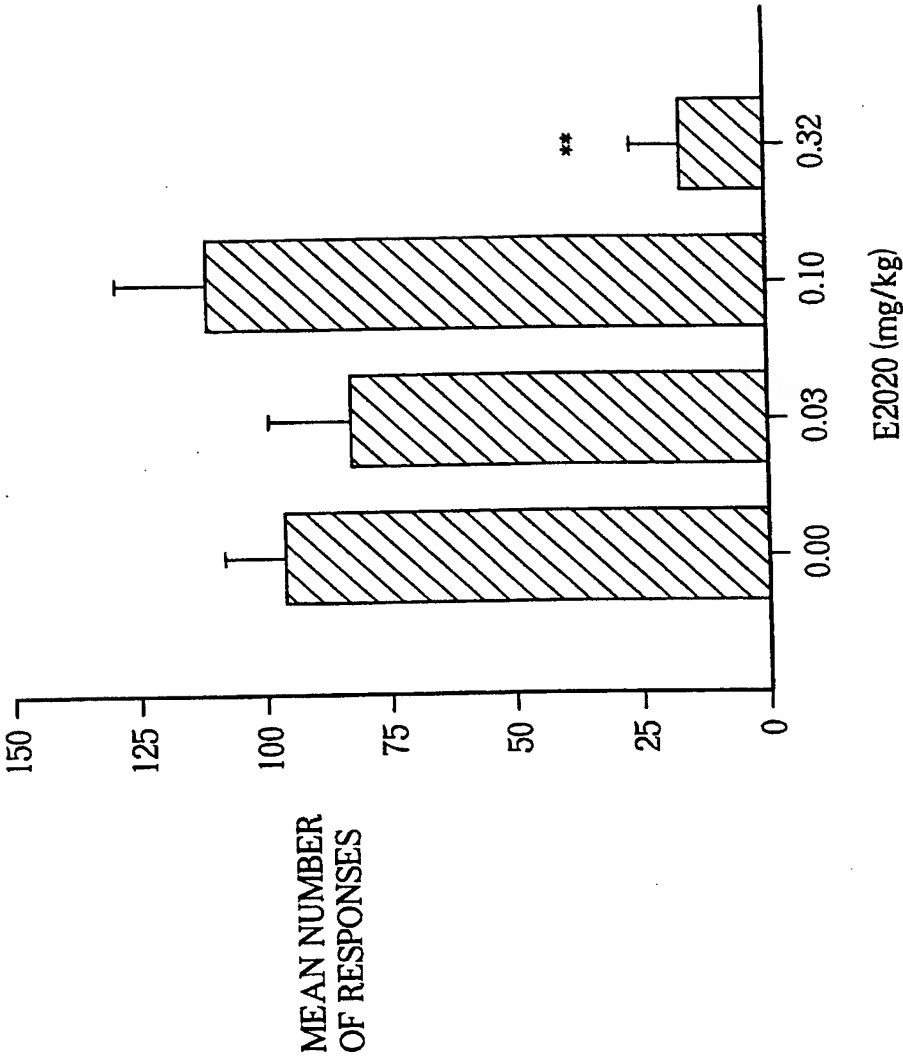
6/10

FIG-6 TACRINE PRODUCED A DECREASE IN RESPONSES ON A CONTINUOUS PERFORMANCE TASK IN RHESUS MONKEYS IN THE ABSENCE OF ANY OBSERVABLE SIDE EFFECTS (N = 6/dose)



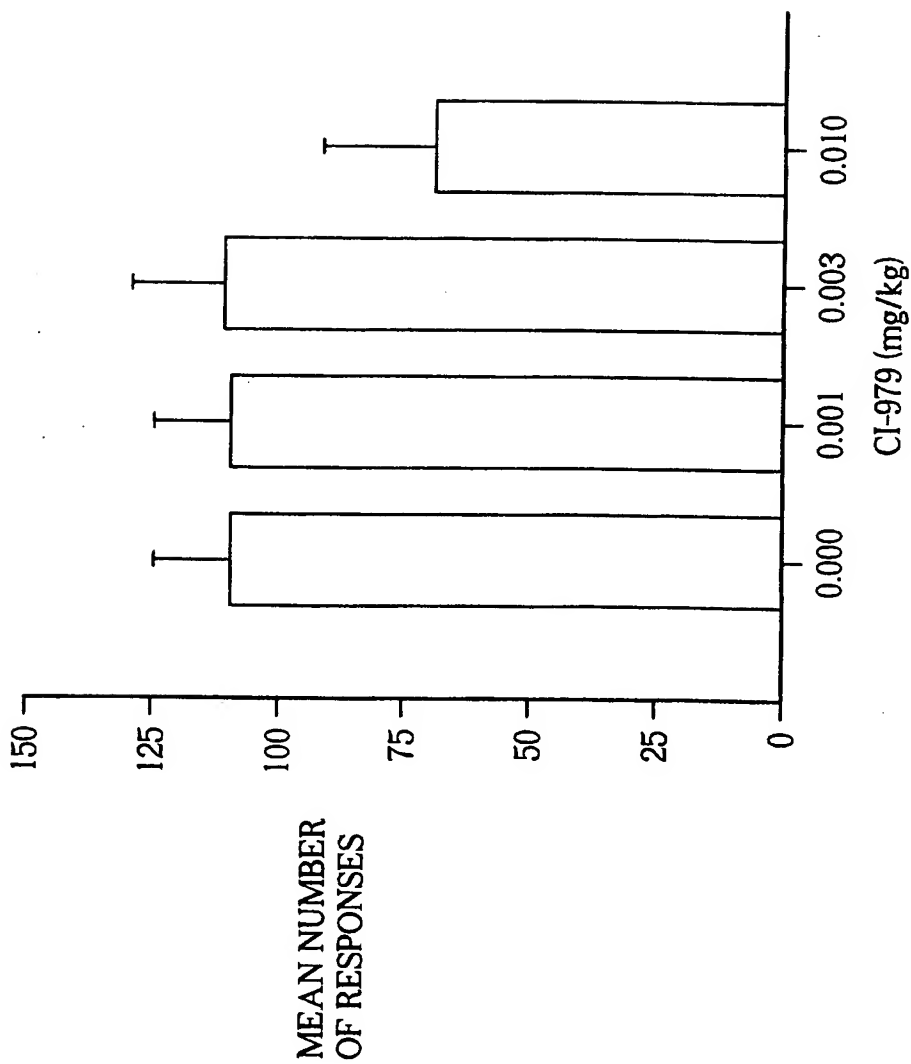
7/10

FIG-7



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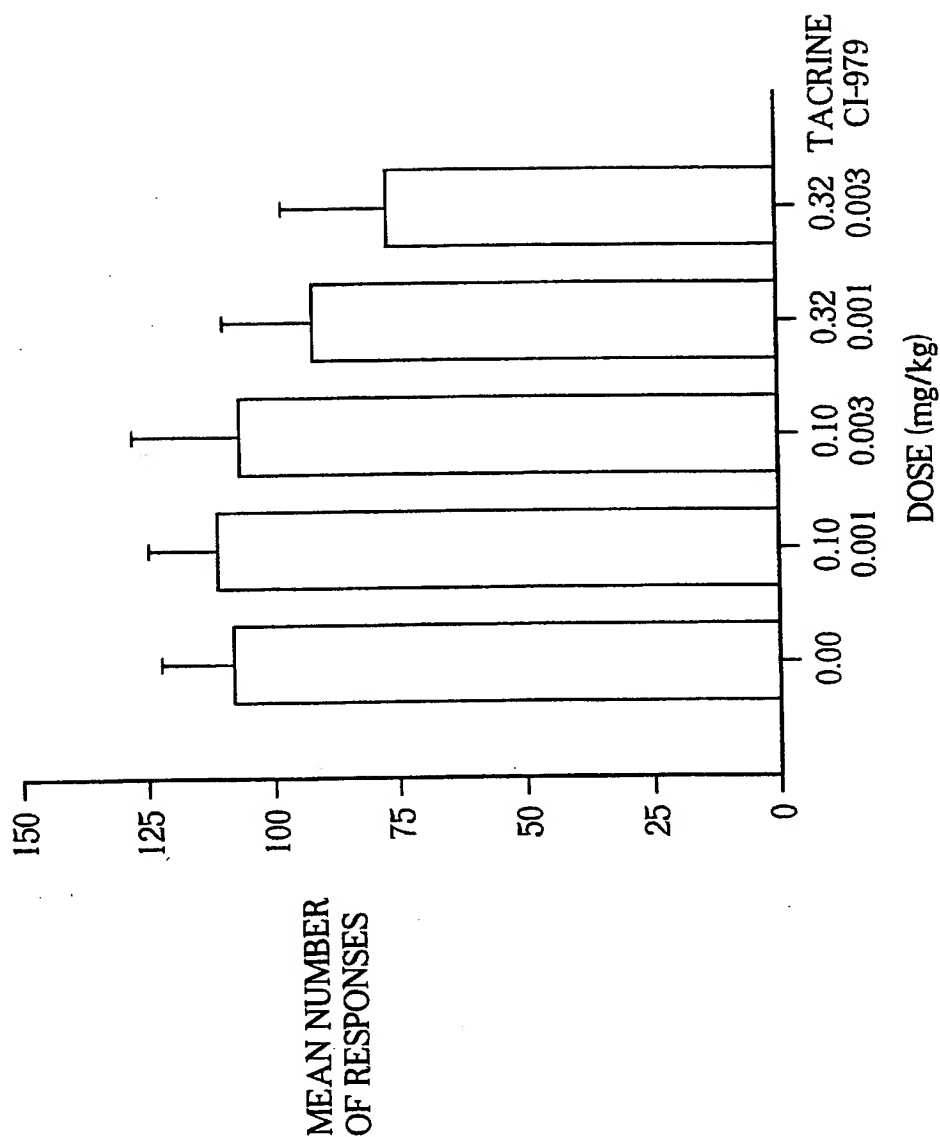
CI-979 AT 0.010 mg/kg PRODUCED A DECREASE IN RESPONSES ON A CONTINUOUS PERFORMANCE TASK IN 3 OF 6 RHESUS MONKEYS WITH 2 MONKEYS EXHIBITING OBVIOUS SALIVATION AND EMESIS (N = 6/dose)

FIG-8

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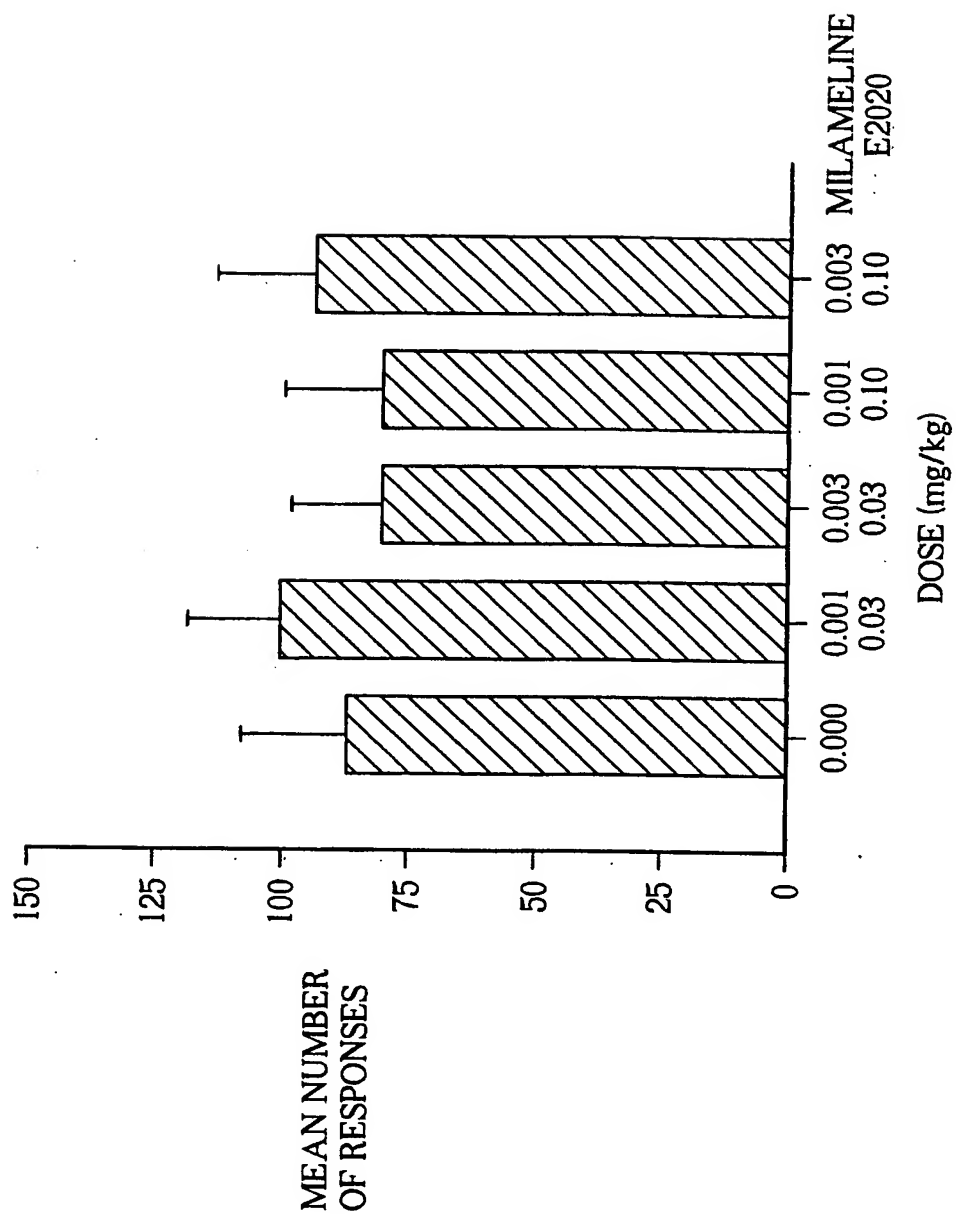
TACRINE AND CI-979 GIVEN IN COMBINATION PRODUCED A SMALL DECREASE IN RESPONSES ON A CONTINUOUS PERFORMANCE TASK IN RHESUS MONKEYS AND NO OBVIOUS POTENTIATION OF PERIPHERAL SIDE EFFECTS (N = 6/dose)

FIG-9



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FIG-10



INTERNATIONAL SEARCH REPORT

International application No
PCT/US 97/23792

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K45/06 A61K31/645 A61K31/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 855 290 A (FISHER ABRAHAM ET AL) 8 August 1989	1,2,12, 13, 20-25, 32,33
A	see column 7, line 39-40; claims 45-49 see column 8, line 3-5	4,6
X	--- FLOOD: "Effect of Acute Arecoline, Tacrine, and Arecoline + Tacrine Post-Training Administration on Retention in Late Middle-Aged Mice" JOURNAL OF GERONTOLOGY, vol. 43, no. 2, 1988, pages B54-B56, XP002064273	1,2,12, 13, 20-25, 32,33
A	see page B54, left-hand column see page B54, right-hand column, line 9-10 see page B56 --- -/-	4

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

7 May 1998

Date of mailing of the international search report

27.05.98

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Authorized officer

Kanbier, D

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US 97/23792

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 2 171 906 A (CIBA GEIGY AG) 10 September 1986 see page 1, line 69-78 see page 2, line 34-37; claims 9-12 ---	1, 12, 20-24, 32
X	WO 96 00062 A (INTERNEURON PHARMA) 4 January 1996 see page 11, line 20-24; claims 1, 4, 6 ---	1, 12, 20-24, 32
A	WO 89 09600 A (MASSACHUSETTS INST TECHNOLOGY) 19 October 1989 see page 4, line 27 - page 5, line 6; claims 1, 3, 6 ---	1, 2, 12, 13, 20, 24, 25, 32, 33
A	US 4 647 580 A (ROSZKOWSKI ADOLPH P) 3 March 1987 see column 2, line 4-11 ---	1
A	DE 41 36 288 A (TROPONWERKE GMBH & CO KG) 6 May 1993 see claim 1 ---	1, 12, 20, 24, 32
P, X	US 5 622 976 A (TAKASUGI HISASHI ET AL) 22 April 1997 see claims -----	1, 12, 20, 24, 32

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 97/ 23792

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 20-39
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 20-39 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US 97/23792

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US 97/23792

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